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Rapeseed lipids to decrease saturated fatty acids in milk and ruminal methane emissions of dairy cows

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Introduction

Ruminants are often criticized for the production of enteric methane that is a potent greenhouse gas. It is estimated that 14 to 17% reduction in greenhouse gas emissions of dairy cows is achievable in developed OECD countries by improving energy efficiency and including lipid supplements into the diet (Mottet *et al.,* 2017). Unsaturated lipids like oil from domestic rapeseeds (*Brassica napus*) have also great potential to modify lipid composition of ruminant meat and milk by decreasing the proportion of saturated fatty acids and increasing that of unsaturated fatty acids inherent to lipid supplements. This is noteworthy as milk and dairy products contribute significantly to human 12:0, 14:0 and 16:0 consumption, excessive intake of these saturated fatty acids being associated with increased risk for cardiovascular disease as well as lowered insulin sensitivity (review by Shingfield *et al*., 2013). The form of lipid inclusion in the dairy cow diet affects bioavailability and final product composition. Milling of rapeseeds was necessary to make lipids within seeds available to animals (Kairenius *et al*., 2009). Furthermore, milled rapeseeds in the diet resulted in a similar fatty acid profile in bovine milk as free rapeseed oil with the exception of lower increase in *trans* fatty acids. The aim of this study was therefore to examine the effects of milled rapeseed on milk fat composition and ruminal methane emissions of dairy cows on grass silage based diets of high digestibility.

Materials and Methods

The study was conducted at the University of Helsinki, Viikki research farm in Finland from the beginning of September to mid November 2018. The whole Finnish Ayrshire herd in milk was fed a control diet for 3 weeks (Period 1) followed by rapeseed lipid-rich diet of 4 weeks (Period 2). After this, all cows were switched back to the control diet (3 weeks, period 3). The dairy herd of Helsinki University was mainly autumn-calving and the number of cows in milk was 49, 52, 50 and 59 at the beginning of the experiment and of sampling Period 1, 2 and 3, respectively. Days in milk were on average 176, 153, 141 and 117 at the beginning of the experiment and of sampling periods 1, 2 and 3, respectively. Forage-rich dairy cow total mixed rations (TMR) based on high quality grass silage were fed *ad libitum* (Table 1). The pre-wilted grass silage was of 1st cut and ensiled with formic acid based additive in big bales. Concentrates in TMR comprised of home-grown cereals, rapeseed feeds as protein supplement, molassed sugar beet pulp and vitamins and minerals. Rapeseed protein was isonitrogenously supplied either as a lipid extracted meal (control diet) or full-fat seeds milled using an ordinary hammer mill (sieve pore size 6 to 8 mm) (test diet). The amount of additional rapeseed lipids in the test diet was adjusted to ca. 50 g/kg diet dry matter (DM). The experimental rapeseed was cultivated in southern Finland and contained 432 total fat, 240 crude protein and 164 neutral detergent fibre (analysed with amylase and expressed inclusive of residual ash) per g/kg DM. Cereal in the control diet was barley and in the test diet oats. The feeding troughs (Insentec RIC, Marknesse, The Netherlands) registered TMR intakes automatically and individually. When visiting the milking-robot, cows producing less than 30, between 30 and 40 and over 40 kg of milk per day at the beginning of the trial received 3, 4 or 5 kg of standard concentrate (Maituri 10000, Raisioagro Ltd, Raisio, Finland) per day throughout the study.

Table 1 The composition of total mixed rations

Ingredient, kg/t dry matter	Control	Test
Grass silage*	600	600
Barley	189	-
Oats	-	136
Rapeseed meal	120	34
Milled rapeseeds	-	139
Molassed sugar beet pulp	70	70
Minerals and vitamins	17	17
Propylene glycol	4	4
Total	1000	1000

*Digestible organic matter 696 g/kg DM

Milk from 13 multiparous dairy cows was individually sampled during the last week of each experimental period for analysis of major milk constituents. Of these 13 cows, 10 were in late lactation (days in milk from 153 to 308 at the beginning of the experiment) and 3 were in early lactation (days in milk from 13 to 27 at the beginning of the experiment). All cows in the herd freely visited a milking robot (Lely Astronaut A3, Lely, Maassluis, The Netherlands) equipped with GreenFeed system (C-Lock Inc., Rapid City, SD, USA) that measures ruminal methane, carbon dioxide and hydrogen emissions. Only records of cows (n=23) that were in milk during all 3 experimental periods and had on average 10 or more accepted reads from GreenFeed in the last week of each experimental period were used for statistical testing. In addition, tank milk was analysed at the end of periods 1 and 2 for fatty acid composition. Chemical composition of feeds and milk were determined as described by Lamminen *et al.* (2019).

Data were analysed by ANOVA for linear and quadratic responses using the Mixed procedure of SAS (version 9.4, 2012). The statistical model contained period as fixed and cow as random effect. The results were considered as statistically significant when $P < 0.05$.

Results and Discussion

The animals had no health concerns when fed the test diet. Animal performance of 13 multiparous dairy cows individually sampled for milk is presented in Table 2. Dry matter intake was decreased by 0.9 kg in test diet relative to control diet ($P < 0.05$ for quadratic response). This is not unexpected as, lipid supplementation often suppresses DM intake at high inclusion rates (Huhtanen *et al*., 2008; Halmemies-Beauchet-Filleau *et al*., 2017). Milk yield linearly decreased throughout the experiment ($P < 0.01$). This can be attributed to advances in the lactation stage of animals as 10 out of 13 were in the late lactation at the beginning of the trial and thus on the descending part of the lactation curve. Indeed, after that effect of time on milk yield was taken into account, no difference in milk yield was seen between test and control diets (mean response of milk yield to test diet just above 0 kg/d). The same was true for energy corrected milk production. Milk fat concentration and fat yield were unaffected by dietary changes. Milk protein yield was linearly decreased during the experiment ($P < 0.01$), but in the absence of quadratic responses, this was most probably related to decreased milk yield due to advances in lactation stage rather than changes in energy and protein status and utilization due to dietary change. Overall, the test diet had no major effect on milk production and major milk constituents. This is in line with previous studies with milled rapeseeds (Kairenius *et al.*, 2009; Brask *et al.,* 2013).

Table 2 Animal performance (n=13)

	Treatment			Mean		Significance	
	Control diet$_{1per}$	Test diet$_{2per}$	Control diet$_{3per}$	response to test diet*	SEM	Lin	Quad
Dry matter intake, kg/d	21.9	21.2	22.2	-0.9	0.70	0.582	0.027
Milk							
Yield, kg/d	31.4	29.7	27.6	0.2	2.66	<0.001	0.638
Energy corrected milk, kg/d	32.4	30.8	29.0	0.1	2.61	0.025	0.796
Fat yield, g/d	1317	1265	1181	16	113.0	0.123	0.727
Fat content, g/kg	42.8	43.3	43.6	0.1	2.27	0.726	0.996
Lactose yield, g/d	1413	1342	1231	20	132.6	<0.001	0.418
Lactose content, g/kg	44.5	44.7	44.1	0.4	0.60	0.216	0.156
Protein yield, g/d	1129	1049	1027	-29	82.8	0.007	0.488
Protein content, g/kg	36.4	36.3	38.1	-1.0	1.03	0.002	0.010
Urea content, mg/dL	27.3	27.0	24.9	0.9	1.37	0.028	0.254

* Test diet$_{2per}$ – (Control diet$_{1per}$ + Control diet$_{3per}$)/2, per = experimental period.

Test diet altered milk fat composition (Table 3). The total saturated fatty acid content of milk fat from the test diet was 17% lower than from the control diet (Table 4). Moreover, the 10- to 16-carbon saturated fatty acids, regarded as the key blood cholesterol-increasing fatty acids in humans, were 31 to 49% lower in milk from the test than in the milk from the control diet. This was expected as increased supply of long-chain fatty acids is known to inhibit de novo synthesis of medium-chain saturates in the mammary gland (review of Shingfield *et al.,* 2010). The total *cis*-monounsaturated fatty acids were 58% higher in milk fat from the test diet than the control diet. These changes principally originate from oleic acid (*cis*-9 18:1) that is the predominant fatty acid in rapeseed. The increase in *trans* fatty acids was marginal and the major *trans* isomers in milk were vaccenic acid (*trans*-11 18:1) and rumenic acid (*cis*-9,*trans*-11 18:2) with potentially beneficial effects on human health (reviews of Field *et al.,* 2009 and Koba *et al.,* 2012).

Table 3 Composition of tank milk collected from the whole herd at the end of experimental periods 1 and 2

Fatty acid, g/100 g total fatty acids	Control diet	Test diet	Change in %
4:0	3.2	3.4	+7
6:0	2.2	1.8	-20
8:0	1.5	1.0	-34
10:0	3.9	2.0	-49
12:0	4.6	2.2	-52
14:0	13	8.5	-35
16:0	31	21	-31
18:0	9.7	18	+82
cis-9 18:1	16	28	+70
trans-11 18:1	1.1	1.2	
total *trans* 18:1	2.4	3.8	
cis-9,*cis*-12 18:2	1.3	1.1	
cis-9,*trans*-11 18:2 (CLA)	0.4	0.5	
cis-9,*cis*-12,*cis*-15 18:3	0.4	0.4	
Total saturated fatty acids	74	61	-17
Total monounsaturated fatty acids	23	36	+58
Total polyunsaturated fatty acids	2.6	2.3	
Total *trans* fatty acids	3.6	5.0	

Ruminal methane, carbon dioxide and hydrogen emissions were substantially decreased from cows on the test diet ($P < 0.01$ for quadratic response, Table 4). For methane and hydrogen, the decrease on the test diet was substantial being 18 and 36% relative to control diet, respectively, whereas for carbon dioxide, the decrease was much smaller (5%). Thus milled rapeseeds seemed to decrease hydrogen load in the rumen. The decrease in methane production in response to rapeseed lipids was slightly higher in magnitude compared to the study of Brask *et al.* (2013, 2-3% added lipids, minus 14%), whereas Martin *et al.* (2011, 3% added lipids) reported no effect. A meta-analysis by Beauchemin *et al.* (2008) showed a linear relationship between percentage of lipid added and reduction in ruminal methane production. Therefore, the high lipid inclusion rate (ca. 5% added lipid) in the present study together with a high-forage diet may at least in part explain the big decrease in ruminal methane emissions in the current study.

Table 4 Ruminal gas production (n=23)

	Treatment			Mean		Significance	
	Control diet$_{1per}$	Test diet$_{2per}$	Control diet$_{3per}$	response to test diet*	SEM	Lin	Quad
Methane, g/d	456	378	468	-84	16.9	0.783	<0.001
Carbon dioxide, g/d	12447	11870	12575	-641	352.8	0.735	<0.001
Hydrogen, mg/d	653	399	598	-227	49.6	0.157	<0.001

* Test diet$_2$per – (Control diet$_1$per + Control diet$_3$per)/2 , per = experimental period

Conclusions

Replacing rapeseed meal with milled rapeseed in a dairy cow diet had no adverse effects on milk production, but improved milk fat profile by decreasing the proportion of medium-chain saturated fatty acids. In addition, milled rapeseeds substantially reduced ruminal methane and hydrogen production.

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